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Title: Screening and Confirmation of Animal Drug Residues by UHPLC-MS-MS		
Revision: 08	Replaces: CLG-MRM1.07	Effective: 11/05/18

Contents

A. INTRODUCTION

1. Summary of Procedure

   Animal drug residues are extracted from tissue using dispersive SPE. The extracted residues are examined using UHPLC-MS-MS using a triple quadrupole mass spectrometer under electrospray ionization (ESI) conditions.

2. Applicability

   This method is suitable for the screening and confirmation of animal drug residues in bovine, poultry, porcine, caprine, and ovine kidney and muscle tissue as well as equine and fish of the order Siluriformes (catfish) muscle tissues at the levels listed in Tables 18A, 18B, 19A, and 19B in Appendix J.3.

   Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

   Note: This method may be performed using standards/solutions that contain fewer analytes than the method is applicable for, if the excluded analytes will not be included in the reported results.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

   a. Platform shaker - Cat. No. 6010, Eberbach

   b. Centrifuge - Thermo IEC, Sorvall RC-6 capable of 3720 rcf

   c. Balance - Mettler Top Loading Model PB300 Balance capable of weighing 2 ± 0.01 g

   d. Balance Analytical - Mettler Model X-205 Dualrange

   e. Turbovap LV Concentration Workstation - Biotage Corp

   f. C18 BakerBond Octadecyl (C18) 40 µm Prep LC Packing

   g. Centrifuge tubes - Polypropylene (PP), 50 mL, Falcon Part number 352070

   h. Centrifuge tubes - Polypropylene (PP), 15 mL, Falcon Part Number 352096

   i. Whatman Mini-UniPrep Syringless filter vials - VWR 0.2 micron, PVDF, Cat. No. 12000-524.

      Note: Avoid glass if the Mini-UniPrep filter vials are substituted with syringe filters and autosampler vials, and substitutes must be checked for possible retention of analytes.

j. Magnetic stirrer and stirbars, freezer, Class A volumetric flasks, Class A graduated cylinders, Pasteur pipettes, repeating pipettes and tips, beakers, bottles, weigh boats, spatulas, funnels, and bottle top volumetric dispensers.

k. LC vials with screw cap lids - Amber glass, 4 mL, Cat. No. 1232 P 49, Thomas Scientific

l. Plastic screw cap vials - Polypropylene, 4 mL, Cat. No. 1708 H 01, Thomas Scientific

2. Instrumentation

a. Waters UHPLC-MS/MS TQD system with MassLynx operating software.

b. UHPLC column - Waters UHPLC HSS T3, 2.1 x 100 mm, 1.8 µm column with VanGuard Precolumn UHPLC HSS T3 2.1 x 5.0 mm, 1.8 µm.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted. The maximum length of time that a working reagent shall be used is 1 year unless the laboratory has produced extension data.

1. Reagents

a. Hexane - HPLC Grade, Fisher Optima Cat. No. H303-4

b. Acetonitrile (ACN) - HPLC Grade, Spectrum Chemical Co. Cat. No. HP412

c. Formic acid - Sigma Chemical Co., Cat. No. F0507-500ML

d. Water - LC Grade, House deionized water passed through an ELGA Pure Lab Ultra Filtration System.

e. Sodium hydroxide (NaOH) - pellets, Fisher, Item No. S318-3.

f. Methanol – Fisher Scientific – Cat. No. A456-4

g. Dimethyl Sulfoxide (DMSO) - Spectrum Chemical – Cat. No. HP412

2. Solutions

a. 80:20 Acetonitrile/Water:

Measure 800 mL of acetonitrile, and 200 mL of deionized water using graduated cylinders and combine. Mix this solution and transfer to a dispenser bottle.

b. 50:50 Acetonitrile/Methanol:

Add equal volumes of acetonitrile and methanol and mix.

c. Hexane (saturated with acetonitrile):

Add 40 mL of acetonitrile to 1 L of hexane in a separatory funnel. Mix this

solution vigorously. Allow the layers to separate and discard the acetonitrile layer. Transfer the saturated hexane to a dispenser bottle for storage. Mix this solution prior to use each day.

d. 0.1% Formic Acid in water:

Add 1.0 mL of formic acid to a 1 L volumetric flask. Dilute to volume with deionized water.

e. 0.03 M Sodium Hydroxide:

Add 0.12 g of NaOH to a 100 ml volumetric flask containing 80 mL of deionized water. Mix and allow solution to cool. Adjust to final volume using deionized water. Store in a plastic container.

f. UHPLC Aqueous Mobile Phase (5% ACN, 95% Water, 0.1% Formic Acid):

Measure 50 mL of acetonitrile and add to a 1 L volumetric flask. Add 1.0 mL of formic acid to the flask. Bring to volume with deionized water. Mix and transfer to the aqueous reservoir of the LC.

g. UHPLC Organic Mobile Phase (Acetonitrile, 0.1% Formic Acid):

Add 1.0 mL of formic acid into a 1 L volumetric flask. Bring to volume using acetonitrile. Mix and transfer to the organic reservoir of the LC.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method. The maximum length of time that an in-house prepared standard shall be used is 1 year unless the laboratory has produced extension data.

1. Standard Information

Table 1 – Standard information

Standard Analyte	Manufacturer	Catalog number	Standard Analyte	Manufacturer	Catalog number
2-Aminosulfone Albendazole	Toronto Research Co.	A580950	Metronidazole - OH	Sigma Aldrich	34007-10MG-R
2-amino-Flubendazole	Sigma Aldrich	32841-10MG	Morantel tartrate	Sigma Aldrich	M5529-25G
2-Quinoxaline Carboxylic Acid (QCA)	Absolute Standards	91819	Moxidectin	Sigma Aldrich	33746-25MG
Abamectin	Sigma Aldrich	31732-100MG	Nafcillin	US Pharmacopeia	1450007
Acepromazine	Sigma Aldrich	A7111-50MG	Norfloxacin	Sigma Aldrich	N9890

Standard Analyte	Manufacturer	Catalog number	Standard Analyte	Manufacturer	Catalog number
Albendazole	Sigma Aldrich	A4673-10G	Orbifloxacin	Sigma Aldrich	34041-100MG-R
Amoxicillin	US Pharmacopeia	1031503	Oxacillin	US Pharmacopeia	1481000
Ampicillin	Sigma Aldrich	A1593	Oxyphenylbutazone	Toronto Research	876950
Azaperone	Sigma Aldrich	34223-100MG	Oxytetracycline	Sigma Aldrich	O5875
Butorphanol	Sigma Aldrich	B9156-100MG	Penicillin G	US Pharmacopeia	1502508
Carazolol	Sigma Aldrich	53787-10MG	Phenylbutazone	MP Biochemicals	153567
Cefazolin	Sigma Aldrich	C5020	Pirlimycin	Pfizer1	Not applicable
Chloramphenicol	Sigma Aldrich	31667	Prednisone	Sigma Aldrich	P6254
Chlorpromazine	Sigma Aldrich	C8138-5G	Promethazine	Sigma Aldrich	P4651-25G
Chlortetracycline	US Pharmacopeia	1129007	Propionylpromazine	Sigma Aldrich	P7780-500MG
Cimaterol	Tocris Bioscience	435	Ractopamine	Sigma Aldrich	34198
Ciprofloxacin	US Pharmacopeia	1134313	Ronidazole	Sigma Aldrich	R7635-5G
Clindamycin	Sigma Aldrich	C5269	Salbutamol	Sigma Aldrich	S8260
Cloxacillin	US Pharmacopeia	1142005	Sarafloxacin	Abbott Labs5	Not applicable
Danofloxacin	Pfizer1	Not applicable	Selamectin	Sigma Aldrich	32476-10MG
DCCD	Pfizer1	Not applicable	Sulfachloropyridazine	Sigma Aldrich	46778
Desethylene Ciprofloxacin	Bayer Healthcare2	Not applicable	Sulfadiazine	Sigma Aldrich	S8626
Diclofenac	Sigma Aldrich	D6899-10G	Sulfadimethoxine	Sigma Aldrich	46794
Dicloxacillin	US Pharmacopeia	1189009	Sulfadoxine	US Pharmacopeia	1626500
Difloxacin	Abbott Labs5	Not applicable	Sulfaethoxypyridazine	Fluka	2743
Dimetridazole	Sigma Aldrich	D4025-100G	Sulfamerazine	Sigma Aldrich	S8876
Dimetridazole - OH	Sigma Aldrich	34003-10MG-R	Sulfamethazine	Sigma Aldrich	S6256
Dipyrone	Sigma Aldrich	46232-250MG	Sulfamethizole	Sigma Aldrich	S5632
Doramectin	Sigma Aldrich	33993-100MG-R	Sulfamethoxazole	Sigma Aldrich	S7507
Doxycycline	Sigma Aldrich	D9891-1G	Sulfamethoxypyridazine	Sigma Aldrich	S7257
Emamectin Benzoate	Sigma Aldrich	31733-250 MG	Sulfanitran	Sigma Aldrich	46882
Enrofloxacin	Bayer Healthcare2	Not applicable	Sulfapyridine	Sigma Aldrich	S6252
Eprinomectin	Sigma Aldrich	32526-100MG	Sulfaquinoxaline	Sigma Aldrich	45662
Erythromycin A	Sigma Aldrich	E0774	Sulfathiazole	Sigma Aldrich	S9876
Fenbendazole	Sigma Aldrich	F5396-5G	Tetracycline	US Pharmacopeia	1651009
Fenbendazole	Sigma Aldrich	32544-10MG	Thiabendazole	Sigma Aldrich	T8904-100G

Standard Analyte	Manufacturer	Catalog number	Standard Analyte	Manufacturer	Catalog number
sulphone					
Florfenicol	Sigma Aldrich	F1427	Tildipirosin	Lonza	RS-STD0512
Flubendazole	Sigma Aldrich	34091-100MG	Tilmicosin	Lilly4	Not applicable
Flunixin	US Pharmacopeia	1274607	Tolfenamic Acid	Sigma Aldrich	T0535-5G
Gamithromycin	Hovione Farma3	Not applicable	Triflupromazine	Sigma Aldrich	46976-250MG
Haloperidol	Sigma Aldrich	H1512-5G	Tulathromycin A	Pfizer1	CP-472,295
Ipronidazole	Sigma Aldrich	32173-10MG	Tylosin	Sigma Aldrich	T6134
Ipronidazole - OH	Sigma Aldrich	34004-10MG-R	Tyvalosin	ECO Animal Health	N/A
Ketamine	Sigma Aldrich	K2753-1G	Virginiamycin	Sigma Aldrich	V2753-10MG
Ketoprofen	Sigma Aldrich	K1751-1G	Xylazine	Sigma Aldrich	X1126-5G
Levamisole	Sigma Aldrich	31742-250MG	13C6 Sulfamethazine Phenyl	Sigma Aldrich6	32519 (Vetranal)
Lincomycin	Sigma Aldrich	L6004	d7 Penicillin G	Toronto Research6	B288600
Melengestrol Acetate	MP Biochemicals	158952	Flunixin d3	Sigma Aldrich6	34083 (Vetranal)
Meloxicam	Sigma Aldrich	M3935-100MG	Taleranol (β-Zearalanol)	Sigma Aldrich	Z0417
Metronidazole	Sigma Aldrich	M3761-5G			

[1] - Pfizer, Groton, CT

[2] - Bayer Healthcare, AG Business Group Pharma, PH-GDD-PT, Clinical Supplies Ops, Wuppertal, Germany.

[3] - Hovione FarmaCiencia SA, Sete Casa, Loures, Portugal

[4] - Lilly Corporate Center, Indianapolis, Indiana

[5] - Abbott Labs, Chicago, IL

[6]- Internal Standard (IS) – optional

[7]- Lonza, Schwabenheim, Germany

[8]- ECO Animal Health, London, England

2. Preparation of Standard Solution(s)

   a. Animal Drug Stock Solutions and internal standard stock solutions

      Prepare animal drug stock solutions and internal standard stock solutions at approximately 1.0 mg/mL when adequate material is available. Other concentrations are used based on two criteria:

i. Solubility of the drug in the solvent

ii. Cost and availability of the drug

For each stock solution, calculate the amount of base material needed (ex. accounting for purity, water, and/or counterion content) to prepare at the concentration listed below using the appropriate solvent listed.

Note: When DMSO is referenced in solvent used, dissolve weighed standard with appropriate volume of DMSO and dilute to volume with appropriate solvent.

Table 2 – Stock standard concentrations

Standard Analyte	Category	Solvent used	Stock Standard Concentration (ng/µL)
2-Aminosulfone Albendazole	Acetonitrile Mix	12.5% DMSO in Methanol	1000
2-amino-Flubendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
2-Quinoxaline Carboxylic Acid (QCA)	Acetonitrile Mix	Purchased	15
Abamectin	Acetonitrile Mix	Acetonitrile	1000
Acepromazine	Acetonitrile Mix	Acetonitrile	1000
Albendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Amoxicillin	Beta Lactam Mix	Water	350
Ampicillin	Beta Lactam Mix	Water	250
Azaperone	Acetonitrile Mix	Methanol	1000
Butorphanol	Acetonitrile Mix	Methanol	1000
Carazolol	Acetonitrile Mix	Acetonitrile	1000
Cefazolin	Beta Lactam Mix	Water	400
Chloramphenicol	Acetonitrile Mix	Acetonitrile	1000
Chlorpromazine	Acetonitrile Mix	Acetonitrile	1000
Chlortetracycline	Acetonitrile Mix	Methanol	1000
Cimaterol	Acetonitrile Mix	Acetonitrile	1000
Ciprofloxacin	Acetonitrile Mix	0.03 M NaOH	1000
Clindamycin	Acetonitrile Mix	Acetonitrile	1000
Cloxacillin	Beta Lactam Mix	Water	200
Danofloxacin	Acetonitrile Mix	0.03 M NaOH	1000
DCCD	Beta Lactam Mix	Water	300
Desethylene Ciprofloxacin	Acetonitrile Mix	0.03 M NaOH	300
Diclofenac	Acetonitrile Mix	6% DMSO in Acetonitrile	1000
Dicloxacillin	Beta Lactam Mix	Water	200
Difloxacin	Acetonitrile Mix	50%ACN/MeOH	500
Dimetridazole	Acetonitrile Mix	Acetonitrile	1000
Dimetridazole - OH	Acetonitrile Mix	Acetone	1000
Dipyrone	Acetonitrile Mix	Methanol	1000
Doramectin	Acetonitrile Mix	Acetonitrile	1000
Doxycycline	Acetonitrile Mix	Methanol	1000

Standard Analyte	Category	Solvent used	Stock Standard Concentration (ng/µL)
Emamectin Benzoate	Acetonitrile Mix	Acetonitrile	1000
Enrofloxacin	Acetonitrile Mix	Acetonitrile	500
Eprinomectin	Acetonitrile Mix	Acetonitrile	1000
Erythromycin A	Acetonitrile Mix	Acetonitrile	1000
Fenbendazole	Acetonitrile Mix	12.5% DMSO in Methanol	1000
Fenbendazole sulphone	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Florfenicol	Acetonitrile Mix	Acetonitrile	1000
Flubendazole	Acetonitrile Mix	12.5% DMSO in Acetonitrile	1000
Flunixin	Acetonitrile Mix	Methanol	1000
Gamithromycin	Acetonitrile Mix	Acetonitrile	500
Haloperidol	Acetonitrile Mix	Methanol	1000
Ipronidazole	Acetonitrile Mix	Acetonitrile	250
Ipronidazole - OH	Acetonitrile Mix	Acetonitrile	250
Ketamine	Acetonitrile Mix	Milli-Q Water	1000
Ketoprofen	Acetonitrile Mix	Acetonitrile	1000
Levamisole	Acetonitrile Mix	12.5% DMSO in Methanol	1000
Lincomycin	Acetonitrile Mix	50%ACN/MeOH	500
Melengestrol Acetate	Acetonitrile Mix	Acetonitrile	1000
Meloxicam	Acetonitrile Mix	Acetonitrile	1000
Metronidazole	Acetonitrile Mix	Acetonitrile	1000
Metronidazole - OH	Acetonitrile Mix	Acetonitrile	500
Morantel tartrate	Acetonitrile Mix	Milli-Q Water	1000
Moxidectin	Acetonitrile Mix	Acetonitrile	1000
Nafcillin	Beta Lactam Mix	Water	300
Norfloxacin	Acetonitrile Mix	Acetonitrile	1000
Orbifloxacin	Acetonitrile Mix	Methanol	500
Oxacillin	Beta Lactam Mix	Water	200
Oxyphenylbutazone	Acetonitrile Mix	Acetonitrile	1000
Oxytetracycline	Acetonitrile Mix	Methanol	1000
Penicillin G	Beta Lactam Mix	Water	250
Phenylbutazone	Acetonitrile Mix	Acetonitrile	1000
Pirlimycin	Acetonitrile Mix	50%ACN/MeOH	1000
Prednisone	Acetonitrile Mix	Methanol	1000
Promethazine	Acetonitrile Mix	Acetonitrile	1000
Propionylpromazine	Acetonitrile Mix	Acetonitrile	1000
Ractopamine	Acetonitrile Mix	Water	1000
Ronidazole	Acetonitrile Mix	Acetonitrile	1000
Salbutamol	Acetonitrile Mix	Acetonitrile	1000
Sarafloxacin	Acetonitrile Mix	Methanol	1000
Selamectin	Acetonitrile Mix	Acetonitrile	1000
Sulfachloropyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfadiazine	Acetonitrile Mix	Acetonitrile	1000
Sulfadimethoxine	Acetonitrile Mix	Acetonitrile	1000

Standard Analyte	Category	Solvent used	Stock Standard Concentration (ng/µL)
Sulfadoxine	Acetonitrile Mix	Acetonitrile	1000
Sulfaethoxypyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamerazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamethazine	Acetonitrile Mix	Acetonitrile	1000
Sulfamethizole	Acetonitrile Mix	Acetonitrile	1000
Sulfamethoxazole	Acetonitrile Mix	Acetonitrile	1000
Sulfamethoxypyridazine	Acetonitrile Mix	Acetonitrile	1000
Sulfanitran	Acetonitrile Mix	Acetonitrile	500
Sulfapyridine	Acetonitrile Mix	Acetonitrile	1000
Sulfaquinoxaline	Acetonitrile Mix	Acetonitrile	500
Sulfathiazole	Acetonitrile Mix	Acetonitrile	1000
Tetracycline	Acetonitrile Mix	Methanol	500
Thiabendazole	Acetonitrile Mix	Methanol	1000
Tildipirosin	Acetonitrile Mix	Methanol	1000
Tilmicosin	Acetonitrile Mix	Acetonitrile	1000
Tolfenamic Acid	Acetonitrile Mix	Acetonitrile	1000
Triflupromazine	Acetonitrile Mix	3% DMSO in Acetonitrile	1000
Tulathromycin A	Acetonitrile Mix	Acetonitrile	1000
Tylosin	Acetonitrile Mix	Acetonitrile	1000
Tyvalosin	Acetonitrile Mix	Methanol	1000
Virginiamycin	Acetonitrile Mix	Methanol	250
Xylazine	Acetonitrile Mix	Acetonitrile	1000
Taleranol (β-Zearalanol)	Acetonitrile Mix	Methanol	1000
13C6 Sulfamethazine Phenyl	Internal Standard Mix	Acetonitrile	1000
d7 Penicillin G	Internal Standard Mix	Water	500
Flunixin d3	Internal Standard Mix	Methanol	1000

"Acetonitrile Mix" stock standards will expire in 6 months. "Beta Lactam Mix" stock standards will expire in 2 months. The stock standards of 13C6 Sulfamethazine Phenyl and Flunixin d3 will expire in 6 months. The stock standard of d7 Penicillin G will expire in 2 months.

Note: All standards shall be stored at $\leq$ -10 ºC

Note: Internal standards are optional in this method and can be used to monitor injection sequence performance within a set. For issues observed, such as inconsistent internal standard area counts, samples may be reinjected or reanalyzed as needed. If internal standards are not used, appropriate volumes and chemicals must be adjusted as outlined in appropriate method steps below.

b. Intermediate standard solutions

Prepare individual intermediate standard solutions as described for the analytes below in 10 mL volumetric flasks. Intermediate stock standards will expire in 6

months when stored at ≤ -10 ºC.

Table 3 – Intermediate standard solutions

Analyte	Stock Standard Concentration (ng/µL)	Solvent used	Volume Stock Standard (µL)	Intermediate Standard Concentration (ng/µL)
Cimaterol	1000	Acetonitrile	500	50
Ractopamine	1000	Acetonitrile	500	50
Salbutamol	1000	Acetonitrile	500	50
Chloramphenicol	1000	Acetonitrile	500	50
Flunixin	1000	Acetonitrile	1000	100
Taleranol (β-Zearalanol)	1000	Acetonitrile	500	50
Melengestrol Acetate	1000	Acetonitrile	500	50

c. Antibiotic drug composite working (spiking) and internal standard (spiking) solutions

i. Prepare the composite “Acetonitrile Mix” working solution(s) for the veterinary drugs contained in the acetonitrile spiking solutions using the stock and intermediate standard solutions above and the volumes listed in the tables below.

(a) Calculate or use the volume of stock or intermediate stock solution required to give the concentration listed for each standard type in the table below.

(b) Pipet the calculated volume of stock into a 10 mL volumetric flask.

(c) Dilute to 10 mL volume with acetonitrile.

(d) Cap flask and mix.

(e) Transfer solution into amber glass LC vials with screw cap lids.

(f) Composite working solutions will expire in 6 months when stored at ≤ -10 ºC.

Table 4A – "Acetonitrile Mix" kidney screening working solution preparation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney screening working solution preparation Kidney Screening Volume (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	1000	12.5	12.5	12.5	12.5	25
2-amino-Flubendazole	1000	5	20	10	10	5
2-Quinoxaline Carboxylic Acid (QCA)	15			1000		2000
Abamectin	1000	12.5	25	12.5	12.5	12.5
Acepromazine	1000	2	2	2	0.5	0.5
Albendazole	1000	12.5	12.5	12.5	12.5	12.5
Azaperone	1000	2	0.5			
Butorphanol	1000	2	0.5		1	2
Carazolol	1000	0.5	0.5	2	1	0.5
Chloramphenicol	50	60	60	60	60	60
Chlorpromazine	1000					1
Chlortetracycline	1000	500	500	500	500	500
Cimaterol	50					
Ciprofloxacin	1000	12.5	12.5		12.5	50
Clindamycin	1000	25	25	25	25	25
Danofloxacin	1000		12.5	25	12.5	12.5
Desethylene Ciprofloxacin	300	41.7	41.7	166.7	41.7	83.3
Diclofenac	1000	2.5	2.5	2.5	5	2.5
Difloxacin	500	25	25	25	25	25
Dimetridazole	1000					
Dimetridazole - OH	1000	100				
Dipyrone	1000					
Doramectin	1000	15		15		
Doxycycline	1000	12.5	12.5	12.5	12.5	
Emamectin Benzoate	1000	15	15	3.75	3.75	3.75
Enrofloxacin	500	25	25	25	25	25
Eprinomectin	1000	7.5	15	15	15	
Erythromycin A	1000	25	25	25	25	25
Fenbendazole	1000	100	100	100	100	100
Fenbendazole sulphone	1000	100	100	100	100	100
Florfenicol	1000	50	50	50	50	50
Flubendazole	1000	5	5	5	5	5
Flunixin	100	62.5	62.5	62.5	62.5	62.5
Gamithromycin	500	50	50	50	50	
Haloperidol	1000	0.5	0.5	1	0.5	0.5
Ipronidazole	250	4	8		4	8
Ipronidazole - OH	250		8			
Ketamine	1000		20			
Ketoprofen	1000	2.5	2.5	10	10	10
Levamisole	1000	25	25	25	25	25
Lincomycin	500	50	50	50	50	50

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney screening working solution preparation Kidney Screening Volume (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Melengestrol Acetate	50	200	200	200	400	400
Meloxicam	1000	5	5	5	5	5
Metronidazole	1000	1	2	2	2	
Metronidazole - OH	500	4	4	4	8	16
Morantel tartrate	1000	175	175	175	175	175
Moxidectin	1000	15		3.75	15	
Norfloxacin	1000	12.5	12.5	12.5	50	12.5
Orbifloxacin	500	25	25	25	25	25
Oxyphenylbutazone	1000	50	25			
Oxytetracycline	1000	500	500	250	250	250
Phenylbutazone	1000	25	25			
Pirlimycin	1000	125	125	125	125	125
Prednisone	1000	100	25		50	
Promethazine	1000	2	2		2	2
Propionylpromazine	1000	2	2		0.5	0.5
Ractopamine	50	150	150	150	150	150
Ronidazole	1000					
Salbutamol	50	30	30	30	30	30
Sarafloxacin	1000	12.5	12.5	12.5	12.5	12.5
Selamectin	1000					
Sulfachloropyridazine	1000	25	25	25	25	25
Sulfadiazine	1000	25	25	25	25	25
Sulfadimethoxine	1000	25	25	25	25	25
Sulfadoxine	1000	25	25	25	25	25
Sulfaethoxypyridazine	1000	25	25	25	25	25
Sulfamerazine	1000	25	25	25	25	25
Sulfamethazine	1000	25	25	25	25	25
Sulfamethizole	1000	25	25	25	25	25
Sulfamethoxazole	1000	25	25	25	25	25
Sulfamethoxypyridazine	1000	25	25	25	25	25
Sulfanitran	500	50	200	200	200	50
Sulfapyridine	1000	25	25	25	25	25
Sulfaquinoxaline	500	50	50	50	50	50
Sulfathiazole	1000	25	25	25	25	25
Tetracycline	500	500	500	500	500	500
Thiabendazole	1000	25	25	25	25	25
Tildipirosin	1000	1000	1000	250	250	
Tilmicosin	1000	30	120	30	30	
Tolfenamic Acid	1000	12.5	25	50	25	12.5
Triflupromazine	1000				2	
Tulathromycin A	1000	500	2000	500		
Tylosin	1000	50	50	50	50	50
Tyvalosin	1000	12.5	12.5	12.5	12.5	12.5

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney screening working solution preparation Kidney Screening Volume (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Virginiamycin	250	100	100	100	100	100
Xylazine	1000		0.5	2		

Table 4B – "Acetonitrile Mix" kidney confirmation working solution preparation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney confirmation working solution preparation Kidney Confirmation Level (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	1000	12.5	25	12.5		50
2-amino-Flubendazole	1000	5	20	20	10	10
2-Quinoxaline Carboxylic Acid (QCA)	15			1000		2000
Abamectin	1000	12.5	25	12.5	25	50
Acepromazine	1000					2
Albendazole	1000	12.5	12.5	12.5	12.5	12.5
Azaperone	1000					
Butorphanol	1000		1		2	2
Carazolol	1000	0.5	0.5	2	1	1
Chloramphenicol	50					
Chlorpromazine	1000					
Chlortetracycline	1000	500	500	500	500	500
Ciprofloxacin	1000				25	
Clindamycin	1000	25	25	25	25	25
Danofloxacin	1000			25	12.5	50
Desethylene Ciprofloxacin	300					
Diclofenac	1000					
Difloxacin	500	25	25	25	25	25
Dimetridazole	1000					
Dimetridazole - OH	1000					
Dipyrone	1000					
Doramectin	1000					
Doxycycline	1000	12.5				
Emamectin Benzoate	1000	15	15	3.75	3.75	7.5
Enrofloxacin	500	25	25	25	50	25
Eprinomectin	1000	15				
Erythromycin A	1000	25	25	25	25	25
Fenbendazole	1000	100	100	100	100	100
Fenbendazole sulphone	1000	100	100	100	100	100
Florfenicol	1000	50	50	50	50	50
Flubendazole	1000	5	5	10	5	5
Flunixin	100	62.5	62.5	62.5	62.5	62.5
Gamithromycin	500	50		50	50	
Haloperidol	1000	1	1		0.5	2

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney confirmation working solution preparation Kidney Confirmation Level (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Ipronidazole	250		8			
Ipronidazole - OH	250					
Ketamine	1000					
Ketoprofen	1000	2.5	2.5	10	10	10
Levamisole	1000	25	100	25	100	100
Lincomycin	500	50	50	50	50	50
Melengestrol Acetate	50	200	200	200	400	400
Meloxicam	1000	5	5	5	5	5
Metronidazole	1000					
Metronidazole - OH	500					
Morantel tartrate	1000	175	175	175	175	700
Moxidectin	1000	15			15	
Norfloxacin	1000	50	12.5	50	50	12.5
Orbifloxacin	500	25	100			
Oxyphenylbutazone	1000	100				
Oxytetracycline	1000	500	500	250	250	250
Phenylbutazone	1000	25	25			
Pirlimycin	1000	125	125	125	125	125
Prednisone	1000	100	100			
Promethazine	1000		2			2
Ractopamine	50	150				
Ronidazole	1000					
Salbutamol	50	30	30	60	60	30
Sarafloxacin	1000	12.5	25	25	50	12.5
Selamectin	1000					
Sulfachloropyridazine	1000	25	25	25		25
Sulfadiazine	1000	25	25	25	25	25
Sulfadimethoxine	1000	25	25	25	25	25
Sulfadoxine	1000	25	25	25	25	25
Sulfaethoxypyridazine	1000	25	25	25	25	25
Sulfamerazine	1000	25	25	25	25	25
Sulfamethazine	1000	25	50	25	100	100
Sulfamethizole	1000	25	100	50	25	25
Sulfamethoxazole	1000	25	25	100	25	100
Sulfamethoxypyridazine	1000	25	100	25	25	25
Sulfanitran	500	50	200	200	200	50
Sulfapyridine	1000		25	100	50	
Sulfaquinoxaline	500	50	50	50	50	50
Sulfathiazole	1000	25	25	25	25	25
Tetracycline	500	2000	500	500	500	500
Thiabendazole	1000	25	25	50	25	25
Tildipirosin	1000	1000	1000	250	250	
Tilmicosin	1000	30	120	30	30	

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" kidney confirmation working solution preparation Kidney Confirmation Level (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Tolfenamic Acid	1000	25	50	50	25	12.5
Triflupromazine	1000					
Tulathromycin A	1000	500	2000			
Tylosin	1000	50	50	50	50	50
Tyvalosin	1000	12.5	12.5	12.5	12.5	12.5
Virginiamycin	250	100	100	100	100	100
Xylazine	1000					

Table 5A – "Acetonitrile Mix" muscle working solution preparation, for screening

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Minimum Muscle Screening Volume (µL)
2-Aminosulfone Albendazole	1000	12.5
2-amino-Flubendazole	1000	5
2-Quinoxaline Carboxylic Acid (QCA)	15	500
Abamectin	1000	12.5
Acepromazine	1000	1
Albendazole	1000	12.5
Azaperone	1000	0.5
Butorphanol	1000	0.5
Carazolol	1000	0.5
Chloramphenicol	50	30
Chlorpromazine	1000	
Chlortetracycline	1000	500
Cimaterol	50	30
Ciprofloxacin	1000	12.5
Clindamycin	1000	25
Danofloxacin	1000	12.5
Desethylene Ciprofloxacin	300	41.7
Diclofenac	1000	2.5
Difloxacin	500	25
Dimetridazole	1000	1
Dimetridazole - OH	1000	25
Dipyrone	1000	12.5
Doramectin	1000	15
Doxycycline	1000	12.5
Emamectin Benzoate	1000	3.75
Enrofloxacin	500	25
Eprinomectin	1000	15
Erythromycin A	1000	25
Fenbendazole	1000	100
Fenbendazole sulphone	1000	100

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Minimum Muscle Screening Volume (µL)
Florfenicol	1000	50
Flubendazole	1000	5
Flunixin	100	62.5
Gamithromycin	500	50
Haloperidol	1000	0.5
Ipronidazole	250	2
Ipronidazole - OH	250	2
Ketamine	1000	10
Ketoprofen	1000	2.5
Levamisole	1000	25
Lincomycin	500	50
Melengestrol Acetate	50	200
Meloxicam	1000	5
Metronidazole	1000	0.5
Metronidazole - OH	500	4
Morantel tartrate	1000	175
Moxidectin	1000	15
Norfloxacin	1000	12.5
Orbifloxacin	500	25
Oxyphenylbutazone	1000	25
Oxytetracycline	1000	250
Phenylbutazone	1000	25
Pirlimycin	1000	125
Prednisone	1000	25
Promethazine	1000	
Propionylpromazine	1000	2
Ractopamine	50	150
Ronidazole	1000	0.5
Salbutamol	50	30
Sarafloxacin	1000	12.5
Selamectin	1000	15
Sulfachloropyridazine	1000	25
Sulfadiazine	1000	25
Sulfadimethoxine	1000	25
Sulfadoxine	1000	25
Sulfaethoxypyridazine	1000	25
Sulfamerazine	1000	25
Sulfamethazine	1000	25
Sulfamethizole	1000	25
Sulfamethoxazole	1000	25
Sulfamethoxypyridazine	1000	25
Sulfanitran	500	50
Sulfapyridine	1000	25

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Minimum Muscle Screening Volume (µL)
Sulfaquinoxaline	500	50
Sulfathiazole	1000	25
Tetracycline	500	500
Thiabendazole	1000	25
Tildipirosin	1000	250
Tilmicosin	1000	30
Tolfenamic Acid	1000	12.5
Triflupromazine	1000	
Tulathromycin A	1000	500
Tylosin	1000	50
Tyvalosin	1000	12.5
Virginiamycin	250	100
Xylazine	1000	0.5
Taleranol (β-Zearalanol)	50	120

Table 5B – “Acetonitrile Mix” muscle confirmation working solution preparation

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	“Acetonitrile Mix” muscle confirmation working solution preparation Muscle Confirmation Volume Level (µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
2-Aminosulfone Albendazole	1000	25	12.5	12.5	50	50	12.5	25
2-amino-Flubendazole	1000	10	5	5	10	10	20	20
2-Quinoxaline Carboxylic Acid (QCA)	15			1000	1000	1000	2000	1000
Abamectin	1000		50			50		
Acepromazine	1000							
Albendazole	1000	12.5	25		25	12.5	12.5	12.5
Azaperone	1000		2					
Butorphanol	1000	0.5	1	1		2		
Carazolol	1000	0.5	0.5	0.5	0.5	1	1	2
Chloramphenicol	50							
Chlorpromazine	1000							
Chlortetracycline	1000	2000	500	500		500	1000	2000
Cimaterol	50							
Ciprofloxacin	1000	50	50	25			50	50
Clindamycin	1000	25	25	25	25	25	25	25
Danofloxacin	1000		25	12.5	12.5	12.5		12.5
Desethylene Ciprofloxacin	300							
Diclofenac	1000							10
Difloxacin	500	25	25	25	25	25	25	100
Dimetridazole	1000							
Dimetridazole - OH	1000	25	25	25	25	50	100	100
Dipyrone	1000		25					12.5
Doramectin	1000							

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" muscle confirmation working solution preparation Muscle Confirmation Volume Level (µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Doxycycline	1000		50					
Emamectin Benzoate	1000		15		7.5			7.5
Enrofloxacin	500	25	25	25	50	25	25	25
Eprinomectin	1000							
Erythromycin A	1000	25	25	25	25	50	25	25
Fenbendazole	1000	100	200	100	100	100	100	100
Fenbendazole sulphone	1000	100	100	100	100	100	100	100
Florfenicol	1000	50	50	50	50	50	50	50
Flubendazole	1000	5	5	5	5	5	5	5
Flunixin	100	62.5	62.5	62.5	62.5	62.5	62.5	62.5
Gamithromycin	500	50	100	200	50	50		100
Haloperidol	1000	0.5	1	0.5	1	0.5	1	0.5
Ipronidazole	250		8					
Ipronidazole - OH	250							
Ketamine	1000							
Ketoprofen	1000	2.5	2.5	2.5	5	2.5		5
Levamisole	1000	25	25	25	25	25	100	
Lincomycin	500	50	50	50	50	50	50	50
Melengestrol Acetate	50	200	400	200	800		200	200
Meloxicam	1000	5	5	5	5	5	10	5
Metronidazole	1000							
Metronidazole - OH	500							
Morantel tartrate	1000	175	175	175	175	175		
Moxidectin	1000		15					
Norfloxacin	1000	12.5	12.5	12.5	12.5	25	50	50
Orbifloxacin	500		25	100	50	50	100	
Oxyphenylbutazone	1000							25
Oxytetracycline	1000	250	250	250	250	250	250	250
Phenylbutazone	1000		50					25
Pirlimycin	1000	125	125	125	125	125	125	125
Prednisone	1000	25	50	25	50	50	100	50
Promethazine	1000							
Ractopamine	50	150	150	150	150			
Ronidazole	1000							
Salbutamol	50	30	30	30	30	60	30	30
Sarafloxacin	1000	12.5	12.5	50	25	12.5	50	
Selamectin	1000							
Sulfachloropyridazine	1000	25	25	100	25	25	50	
Sulfadiazine	1000	25	25	25	25	25	25	50
Sulfadimethoxine	1000	25	25	25	25	25	25	25
Sulfadoxine	1000	25	25	25	25	25	25	25
Sulfaethoxypyridazine	1000	50	25	25	25	25	25	25
Sulfamerazine	1000	25	25	25	25	25	50	50

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	"Acetonitrile Mix" muscle confirmation working solution preparation Muscle Confirmation Volume Level (µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Sulfamethazine	1000		25		50	25	100	
Sulfamethizole	1000	25	50	25	25	25	25	100
Sulfamethoxazole	1000	25	25	25	50	25	25	25
Sulfamethoxypyridazine	1000	25	25	25	25	100	50	
Sulfanitran	500	50	50	100	50	50	100	50
Sulfapyridine	1000	25	25	25	25	25		
Sulfaquinoxaline	500	50	50	50	50	50	50	50
Sulfathiazole	1000	25	25	25	25	25	25	25
Tetracycline	500	500	500		500	500	1000	2000
Thiabendazole	1000	25	25	25	50	25		
Tildipirosin	1000	250	250	250	250	250	250	250
Tilmicosin	1000	30	30	30	30	30	30	30
Tolfenamic Acid	1000	25	25	25	50	25	25	50
Triflupromazine	1000							
Tulathromycin A	1000	1000	500	1000		500	1000	2000
Tylosin	1000	50	50	50	50	50	50	50
Tyvalosin	1000	12.5	25	12.5	12.5	12.5	12.5	12.5
Virginiamycin	250	100	100	100	100	100	100	100
Xylazine	1000							
Taleranol (β-Zearalanol)	50							240

Using these volumes yields the following concentrations as found in the tables below:

Table 6A – "Acetonitrile Mix" kidney screening working standard concentrations

Standard Analyte	"Acetonitrile Mix" kidney screening working standard concentrations Kidney Screening Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	1.25	1.25	1.25	1.25	2.5
2-amino-Flubendazole	0.5	2	1	1	0.5
2-Quinoxaline Carboxylic Acid (QCA)			1.5		3
Abamectin	1.25	2.5	1.25	1.25	1.25
Acepromazine	0.2	0.2	0.2	0.05	0.05
Albendazole	1.25	1.25	1.25	1.25	1.25
Azaperone	0.2	0.05			
Butorphanol	0.2	0.05		0.1	0.2
Carazolol	0.05	0.05	0.2	0.1	0.05
Chloramphenicol	0.3	0.3	0.3	0.3	0.3
Chlorpromazine					0.1
Chlortetracycline	50	50	50	50	50
Ciprofloxacin	1.25	1.25		1.25	5
Clindamycin	2.5	2.5	2.5	2.5	2.5
Danofloxacin		1.25	2.5	1.25	1.25

Standard Analyte	"Acetonitrile Mix" kidney screening working standard concentrations Kidney Screening Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Desethylene Ciprofloxacin	1.25	1.25	5	1.25	2.5
Diclofenac	0.25	0.25	0.25	0.5	0.25
Difloxacin	1.25	1.25	1.25	1.25	1.25
Dimetridazole					
Dimetridazole - OH	10				
Dipyrone					
Doramectin	1.5		1.5		
Doxycycline	1.25	1.25	1.25	1.25	
Emamectin Benzoate	1.5	1.5	0.375	0.375	0.375
Enrofloxacin	1.25	1.25	1.25	1.25	1.25
Eprinomectin	0.75	1.5	1.5	1.5	
Erythromycin A	2.5	2.5	2.5	2.5	2.5
Fenbendazole	10	10	10	10	10
Fenbendazole sulphone	10	10	10	10	10
Florfenicol	5	5	5	5	5
Flubendazole	0.5	0.5	0.5	0.5	0.5
Flunixin	0.625	0.625	0.625	0.625	0.625
Gamithromycin	2.5	2.5	2.5	2.5	
Haloperidol	0.05	0.05	0.1	0.05	0.05
Ipronidazole	0.1	0.2		0.1	0.2
Ipronidazole - OH		0.2			
Ketamine		2			
Ketoprofen	0.25	0.25	1	1	1
Levamisole	2.5	2.5	2.5	2.5	2.5
Lincomycin	2.5	2.5	2.5	2.5	2.5
Melengestrol Acetate	1	1	1	2	2
Meloxicam	0.5	0.5	0.5	0.5	0.5
Metronidazole	0.1	0.2	0.2	0.2	
Metronidazole - OH	0.2	0.2	0.2	0.4	0.8
Morantel tartrate	17.5	17.5	17.5	17.5	17.5
Moxidectin	1.5		0.375	1.5	
Norfloxacin	1.25	1.25	1.25	5	1.25
Orbifloxacin	1.25	1.25	1.25	1.25	1.25
Oxyphenylbutazone	5	2.5			
Oxytetracycline	50	50	25	25	25
Phenylbutazone	2.5	2.5			
Pirlimycin	12.5	12.5	12.5	12.5	12.5
Prednisone	10	2.5		5	
Promethazine	0.2	0.2		0.2	0.2
Propionylpromazine	0.2	0.2		0.05	0.05
Ractopamine	0.75	0.75	0.75	0.75	0.75
Ronidazole					
Salbutamol	0.15	0.15	0.15	0.15	0.15

Standard Analyte	"Acetonitrile Mix" kidney screening working standard concentrations Kidney Screening Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Sarafloxacin	1.25	1.25	1.25	1.25	1.25
Selamectin					
Sulfachloropyridazine	2.5	2.5	2.5	2.5	2.5
Sulfadiazine	2.5	2.5	2.5	2.5	2.5
Sulfadimethoxine	2.5	2.5	2.5	2.5	2.5
Sulfadoxine	2.5	2.5	2.5	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5	2.5	2.5	2.5
Sulfamerazine	2.5	2.5	2.5	2.5	2.5
Sulfamethazine	2.5	2.5	2.5	2.5	2.5
Sulfamethizole	2.5	2.5	2.5	2.5	2.5
Sulfamethoxazole	2.5	2.5	2.5	2.5	2.5
Sulfamethoxypyridazine	2.5	2.5	2.5	2.5	2.5
Sulfanitran	2.5	10	10	10	2.5
Sulfapyridine	2.5	2.5	2.5	2.5	2.5
Sulfaquinoxaline	2.5	2.5	2.5	2.5	2.5
Sulfathiazole	2.5	2.5	2.5	2.5	2.5
Tetracycline	25	25	25	25	25
Thiabendazole	2.5	2.5	2.5	2.5	2.5
Tildipirosin	100	100	25	25	
Tilmicosin	3	12	3	3	
Tolfenamic Acid	1.25	2.5	5	2.5	1.25
Triflupromazine				0.2	
Tulathromycin A	50	200	50		
Tylosin	5	5	5	5	5
Tyvalosin	1.25	1.25	1.25	1.25	1.25
Virginiamycin	2.5	2.5	2.5	2.5	2.5
Xylazine		0.05	0.2		

Table 6B – "Acetonitrile Mix" kidney confirmation working standard concentrations

Standard Analyte	"Acetonitrile Mix" kidney confirmation working standard concentrations Kidney Confirmation Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	1.25	2.5	1.25		5
2-amino-Flubendazole	0.5	2	2	1	1
2-Quinoxaline Carboxylic Acid (QCA)			1.5		3
Abamectin	1.25	2.5	1.25	2.5	5
Acepromazine					0.2
Albendazole	1.25	1.25	1.25	1.25	1.25
Azaperone					
Butorphanol		0.1		0.2	0.2

Standard Analyte	"Acetonitrile Mix" kidney confirmation working standard concentrations Kidney Confirmation Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Carazolol	0.05	0.05	0.2	0.1	0.1
Chloramphenicol					
Chlorpromazine					
Chlortetracycline	50	50	50	50	50
Ciprofloxacin				2.5	
Clindamycin	2.5	2.5	2.5	2.5	2.5
Danofloxacin			2.5	1.25	5
Desethylene Ciprofloxacin					
Diclofenac					
Difloxacin	1.25	1.25	1.25	1.25	1.25
Dimetridazole					
Dimetridazole - OH					
Dipyrone					
Doramectin					
Doxycycline	1.25				
Emamectin Benzoate	1.5	1.5	0.375	0.375	0.75
Enrofloxacin	1.25	1.25	1.25	2.5	1.25
Eprinomectin	1.5				
Erythromycin A	2.5	2.5	2.5	2.5	2.5
Fenbendazole	10	10	10	10	10
Fenbendazole sulphone	10	10	10	10	10
Florfenicol	5	5	5	5	5
Flubendazole	0.5	0.5	1	0.5	0.5
Flunixin	0.625	0.625	0.625	0.625	0.625
Gamithromycin	2.5		2.5	2.5	
Haloperidol	0.1	0.1		0.05	0.2
Ipronidazole		0.2			
Ipronidazole - OH					
Ketamine					
Ketoprofen	0.25	0.25	1	1	1
Levamisole	2.5	10	2.5	10	10
Lincomycin	2.5	2.5	2.5	2.5	2.5
Melengestrol Acetate	1	1	1	2	2
Meloxicam	0.5	0.5	0.5	0.5	0.5
Metronidazole					
Metronidazole - OH					
Morantel tartrate	17.5	17.5	17.5	17.5	70
Moxidectin	1.5			1.5	
Norfloxacin	5	1.25	5	5	1.25
Orbifloxacin	1.25	5			
Oxyphenylbutazone	10				
Oxytetracycline	50	50	25	25	25
Phenylbutazone	2.5	2.5			

Standard Analyte	"Acetonitrile Mix" kidney confirmation working standard concentrations Kidney Confirmation Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Pirlimycin	12.5	12.5	12.5	12.5	12.5
Prednisone	10	10			
Promethazine		0.2			0.2
Ractopamine	0.75				
Ronidazole					
Salbutamol	0.15	0.15	0.3	0.3	0.15
Sarafloxacin	1.25	2.5	2.5	5	1.25
Selamectin					
Sulfachloropyridazine	2.5	2.5	2.5		2.5
Sulfadiazine	2.5	2.5	2.5	2.5	2.5
Sulfadimethoxine	2.5	2.5	2.5	2.5	2.5
Sulfadoxine	2.5	2.5	2.5	2.5	2.5
Sulfaethoxypyridazine	2.5	2.5	2.5	2.5	2.5
Sulfamerazine	2.5	2.5	2.5	2.5	2.5
Sulfamethazine	2.5	5	2.5	10	10
Sulfamethizole	2.5	10	5	2.5	2.5
Sulfamethoxazole	2.5	2.5	10	2.5	10
Sulfamethoxypyridazine	2.5	10	2.5	2.5	2.5
Sulfanitran	2.5	10	10	10	2.5
Sulfapyridine		2.5	10	5	
Sulfaquinoxaline	2.5	2.5	2.5	2.5	2.5
Sulfathiazole	2.5	2.5	2.5	2.5	2.5
Tetracycline	100	25	25	25	25
Thiabendazole	2.5	2.5	5	2.5	2.5
Tildipirosin	100	100	25	25	
Tilmicosin	3	12	3	3	
Tolfenamic Acid	2.5	5	5	2.5	1.25
Triflupromazine					
Tulathromycin A	50	200			
Tylosin	5	5	5	5	5
Tyvalosin	1.25	1.25	1.25	1.25	1.25
Virginiamycin	2.5	2.5	2.5	2.5	2.5
Xylazine					

Table 7 – "Acetonitrile Mix" muscle working standard concentrations

Standard Analyte	Minimum Muscle Screening Level (ng/µL)	"Acetonitrile Mix" muscle working standard concentrations Muscle Confirmation Level (ng/µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
2-Aminosulfone Albendazole	1.25	2.5	1.25	1.25	5	5	1.25	2.5
2-amino-Flubendazole	0.5	1	0.5	0.5	1	1	2	2
2-Quinoxaline Carboxylic Acid (QCA)	0.75			1.5	1.5	1.5	3	1.5
Abamectin	1.25		5			5		

Standard Analyte	Minimum Muscle Screening Level (ng/µL)	"Acetonitrile Mix" muscle working standard concentrations Muscle Confirmation Level (ng/µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Acepromazine	0.1							
Albendazole	1.25	1.25	2.5		2.5	1.25	1.25	1.25
Azaperone	0.05		0.2					
Butorphanol	0.05	0.05	0.1	0.1		0.2		
Carazolol	0.05	0.05	0.05	0.05	0.05	0.1	0.1	0.2
Chloramphenicol	0.15							
Chlorpromazine								
Chlortetracycline	50	200	50	50		50	100	200
Cimaterol	0.15							
Ciprofloxacin	1.25	5	5	2.5			5	5
Clindamycin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Danofloxacin	1.25		2.5	1.25	1.25	1.25		1.25
Desethylene Ciprofloxacin	1.25							
Diclofenac	0.25							1
Difloxacin	1.25	1.25	1.25	1.25	1.25	1.25	1.25	5
Dimetridazole	0.1							
Dimetridazole - OH	2.5	2.5	2.5	2.5	2.5	5	10	10
Dipyrone	1.25		2.5					1.25
Doramectin	1.5							
Doxycycline	1.25		5					
Emamectin Benzoate	0.375		1.5		0.75			0.75
Enrofloxacin	1.25	1.25	1.25	1.25	2.5	1.25	1.25	1.25
Eprinomectin	1.5							
Erythromycin A	2.5	2.5	2.5	2.5	2.5	5	2.5	2.5
Fenbendazole	10	10	20	10	10	10	10	10
Fenbendazole sulphone	10	10	10	10	10	10	10	10
Florfenicol	5	5	5	5	5	5	5	5
Flubendazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Flunixin	0.625	0.625	0.625	0.625	0.625	0.625	0.625	0.625
Gamithromycin	2.5	2.5	5	10	2.5	2.5		5
Haloperidol	0.05	0.05	0.1	0.05	0.1	0.05	0.1	0.05
Ipronidazole	0.05		0.2					
Ipronidazole - OH	0.05							
Ketamine	1							
Ketoprofen	0.25	0.25	0.25	0.25	0.5	0.25		0.5
Levamisole	2.5	2.5	2.5	2.5	2.5	2.5	10	
Lincomycin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Melengestrol Acetate	1	1	2	1	4		1	1
Meloxicam	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5
Metronidazole	0.05							
Metronidazole - OH	0.2							
Morantel tartrate	17.5	17.5	17.5	17.5	17.5	17.5		
Moxidectin	1.5		1.5					

Standard Analyte	Minimum Muscle Screening Level (ng/µL)	"Acetonitrile Mix" muscle working standard concentrations Muscle Confirmation Level (ng/µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Norfloxacin	1.25	1.25	1.25	1.25	1.25	2.5	5	5
Orbifloxacin	1.25		1.25	5	2.5	2.5	5	
Oxyphenylbutazone	2.5							2.5
Oxytetracycline	25	25	25	25	25	25	25	25
Phenylbutazone	2.5		5					2.5
Pirlimycin	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Prednisone	2.5	2.5	5	2.5	5	5	10	5
Promethazine								
Ractopamine	0.75	0.75	0.75	0.75	0.75			
Ronidazole	0.05							
Salbutamol	0.15	0.15	0.15	0.15	0.15	0.3	0.15	0.15
Sarafloxacin	1.25	1.25	1.25	5	2.5	1.25	5	
Selamectin	1.5							
Sulfachloropyridazine	2.5	2.5	2.5	10	2.5	2.5	5	
Sulfadiazine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	5
Sulfadimethoxine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sulfadoxine	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sulfaethoxypyridazine	2.5	5	2.5	2.5	2.5	2.5	2.5	2.5
Sulfamerazine	2.5	2.5	2.5	2.5	2.5	2.5	5	5
Sulfamethazine	2.5		2.5		5	2.5	10	
Sulfamethizole	2.5	2.5	5	2.5	2.5	2.5	2.5	10
Sulfamethoxazole	2.5	2.5	2.5	2.5	5	2.5	2.5	2.5
Sulfamethoxypyridazine	2.5	2.5	2.5	2.5	2.5	10	5	
Sulfanitran	2.5	2.5	2.5	5	2.5	2.5	5	2.5
Sulfapyridine	2.5	2.5	2.5	2.5	2.5	2.5		
Sulfaquinoxaline	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Sulfathiazole	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Tetracycline	25	25	25		25	25	50	100
Thiabendazole	2.5	2.5	2.5	2.5	5	2.5		
Tildipirosin	25	25	25	25	25	25	25	25
Tilmicosin	3	3	3	3	3	3	3	3
Tolfenamic Acid	1.25	2.5	2.5	2.5	5	2.5	2.5	5
Triflupromazine								
Tulathromycin A	50	100	50	100		50	100	200
Tylosin	5	5	5	5	5	5	5	5
Tyvalosin	1.25	1.25	2.5	1.25	1.25	1.25	1.25	1.25
Virginiamycin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Xylazine	0.05							
Taleranol (β-Zearalanol)	0.6							1.2

ii. Prepare the composite working solution(s) for the veterinary drugs contained in the "Beta Lactam mix" spiking solutions using the stock standard solutions above and the volumes listed in the table below.

(a) Calculate or use the volume of stock solution required to give the concentrations listed for each standard type in the table below.

(b) Pipet the calculated volume of stock into a 10 mL volumetric flask.

(c) Dilute to 10 mL volume with water.

(d) Cap flask and mix.

(e) Transfer solution into plastic screw cap vials.

(f) Composite working solutions will expire in 2 months when stored at ≤ -10 ºC.

Table 8A – Beta-lactam kidney screening working solutions preparation volumes

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Beta-lactam kidney screening working solutions preparation volumes Kidney Screening Volume (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Amoxicillin	350					
Ampicillin	250	20	20		10	40
Cefazolin	400	62.5	62.5	62.5	62.5	62.5
Cloxacillin	200	25	12.5	12.5	12.5	12.5
DCCD	300	333.3	166.7	333.3	83.3	333.3
Dicloxacillin	200	125	125	125	125	125
Nafcillin	300	83.3	83.3	83.3	83.3	83.3
Oxacillin	200	125	125	125	125	125
Penicillin G	250	50	50	50	50	50

Table 8B – Beta-lactam kidney confirmation working solutions preparation volumes

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Beta-lactam kidney confirmation working solutions preparation volumes Kidney Confirmation Level (µL)				
		Bovine	Porcine	Poultry	Caprine	Ovine
Amoxicillin	350					
Ampicillin	250	20	20		40	40
Cefazolin	400	125	125	125	125	62.5
Cloxacillin	200	25	50		25	50
DCCD	300	333.3	333.3	333.3	166.7	
Dicloxacillin	200	125	125	125	125	125
Nafcillin	300	83.3	83.3	83.3	83.3	83.3
Oxacillin	200	125	125	125	125	125
Penicillin G	250	50	50	50	100	100

Table 9 – Beta-lactam muscle working solutions preparation volume

SStandard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Minimum Muscle Screening Volume (µL)	Beta-lactam muscle working solutions preparation volumes Muscle Confirmation Volume (µL)						
			Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Amoxicillin	350	114.3							
Ampicillin	250	10	20		10		20		20
Cefazolin	400	62.5	125	125	125	125	250	62.5	250
Cloxacillin	200	12.5	25	25	25	12.5	12.5		25
DCCD	300	83.3	166.7	333.3	333.3	333.3		333.3	333.3
Dicloxacillin	200	125	125	125	125	125	125	125	125
Nafcillin	300	83.3	83.3	83.3	83.3	83.3	83.3	83.3	83.3
Oxacillin	200	125	125	125	125	125	125	125	125
Penicillin G	250	50	50	50	50	50	50	100	200

Using these volumes yields the following concentrations as found in the tables below:

Table 10A – Beta-lactam kidney working solution levels for screening

Standard Analyte	Beta-lactam kidney working solution levels for screening Kidney Screening Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Amoxicillin					
Ampicillin	0.5	0.5		0.25	1
Cefazolin	2.5	2.5	2.5	2.5	2.5
Cloxacillin	0.5	0.25	0.25	0.25	0.25
DCCD	10	5	10	2.5	10
Dicloxacillin	2.5	2.5	2.5	2.5	2.5
Nafcillin	2.5	2.5	2.5	2.5	2.5
Oxacillin	2.5	2.5	2.5	2.5	2.5
Penicillin G	1.25	1.25	1.25	1.25	1.25

Table 10B – Beta-lactam kidney confirmation working solution levels

Standard Analyte	Beta-lactam kidney confirmation working solution levels Kidney Confirmation Level (ng/µL)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Amoxicillin					
Ampicillin	0.5	0.5		1	1
Cefazolin	5	5	5	5	2.5
Cloxacillin	0.5	1		0.5	1
DCCD	10	10	10	5	
Dicloxacillin	2.5	2.5	2.5	2.5	2.5
Nafcillin	2.5	2.5	2.5	2.5	2.5
Oxacillin	2.5	2.5	2.5	2.5	2.5
Penicillin G	1.25	1.25	1.25	2.5	2.5

Table 11 – Beta-lactam muscle working solution concentrations

Standard Analyte	Minimum Muscle Screening Level (ng/µL)	Beta-lactam muscle working solution concentrations Muscle Confirmation Levels (ng/µL)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Amoxicillin	4							
Ampicillin	0.25	0.5		0.25		0.5		0.5
Cefazolin	2.5	5	5	5	5	10	2.5	10
Cloxacillin	0.25	0.5	0.5	0.5	0.25	0.25		0.5
DCCD	2.5	5	10	10	10		10	10
Dicloxacillin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Nafcillin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Oxacillin	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Penicillin G	1.25	1.25	1.25	1.25	1.25	1.25	2.5	5

iii. Prepare the composite (spiking) solution for the isotopically-labeled veterinary drugs used for internal standards at a concentration of 20 ng/uL if internal standards will be used. This solution can contain any number of the internal standards listed in the method.

(a) Calculate the volume of stock solution required to give the 20 ng/uL level (see the following table).

(b) Pipet the calculated volume of stock into a 5 mL volumetric flask.

(c) Dilute to 5 mL with acetonitrile.

(d) Cap flask and mix.

(e) Transfer 1.25 mL of solution into 4 amber glass LC vials.

(f) Store at < -10 ºC. The stability of the solution is dependent on which internal standards are present. If the solution contains D7 Penicillin G, then the solution is stable for 2 months. Otherwise, the solution is stable for 6 months.

Table 12 – IS spiking solution

Standard Analyte	Stock or Intermediate Standard Concentration (ng/µL)	Volume (µL)	Final Volume (mL)	Working Standard Concentration (ng/µL)
13C6 Sulfamethazine Phenyl	1000	100	5	20
d7 Penicillin G	500	200	5	20
Flunixin-d3	1000	100	5	20

3. Preparation of External Calibration Curve (Optional)

Use the following table to prepare external standards.

Table 13 – Preparation of external standards

Target Conc.	Volume Acetonitrile Standard Mix (µL)	Volume Beta Lactam Standard Mix (µL)	Volume Internal Standard Mix (µL)	Volume Acetonitrile (µL)	Volume of 0.1% Formic Acid in water
0 X	0	0	20	120	860
1/4 X	10	10	20	110	850
1/2 X	20	20	20	100	840
1 X	40	40	20	80	820
2 X	80	80	20	40	780
3 X	120	120	20	0	740

Note: The volume of acetonitrile will be increased by 20 µL to maintain a total volume of 1 mL if no internal standard solution is added. External standards should be prepared the day of use.

(Optional) Filter external standards with Whatman Mini UniPrep Syringless Filter Vials, PVDF, 0.2 micron.

E. SAMPLE PREPARATION

Samples collected fresh must be kept cold before and during shipping to the laboratory. Once received at the laboratory, samples must be frozen (< -10 °C) prior to grinding if they cannot be prepared on the day of receipt. Once frozen, the sample should be allowed to thaw, while keeping it as cold as possible. Dissect away fat and connective tissue. Grind tissue in blender or vertical cutter-mixer until homogeneous. Store samples frozen (< -10 °C) prior to analysis.

Sample preparation may also be done by dry ice grinding as follows:

a. Chop 0.5 - 1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.

b. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step E.a. may be discarded.

c. For any retained sample, tighten the caps and store in a freezer at -10 ºC.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

a. Weigh 2 ± 0.1 g of homogenized samples into labeled 50 mL polypropylene centrifuge tubes.

Note: Use corresponding blank tissue for controls for each specific species and tissue sample being analyzed.

i. Screening - Weigh one portion each for a blank (negative control), a 1/2 X recovery (positive control), a matrix matched standard, and a check sample, if necessary.

ii. Confirmation - Weigh six 2 g portions of blank tissue into 50 ml polypropylene centrifuge tubes. One for 1/2 X recovery (positive control), one for the blank (negative control) and four for the matrix matched standards (1/2X, 1X, 2X, and 3X). Weigh one additional portion for a check sample, if necessary.

iii. Prepare recoveries, check samples, blank, and samples using the solutions and volumes in the table below:

Table 14 – Preparation of controls and samples

Sample Type	Acetonitrile Standard Mix (µL)	Beta Lactam Standard Mix (µL)	Internal Standard Mix (µL)
Samples and Negative Controls			40
½ X Recovery	40	40	40
1X Recovery	80	80	40
2X Recovery	160	160	40

Note: The "1/2 X Recovery" sample type represents the minimum level of applicability (refer to Section J.3).

2. Extraction Procedure

a. Vortex all uncapped tubes 10 seconds each to mix chemicals with matrix and allow to stand 5 minutes.

Note: Press down to start swirling action slowly and then increase speed to mix without splashing.

b. Add 10.0 mL of 4/1 (v/v) acetonitrile/water to all tubes using a calibrated solvent dispenser. Cap tubes well.

c. Place the racks of tubes in platform shaker on high for 5 minutes.

d. Centrifuge the tubes at >3000 rcf for 5 minutes.

e. Decant each extract into a pre-labeled 50 mL polypropylene centrifuge tubes containing 0.5 g of C18.

f. Add approximately 10 mL of hexane (saturated with acetonitrile) to all tubes. Cap all tubes well.

g. Shake all tubes in the platform shaker on high for 1 minute.

h. Centrifuge all tubes at approximately 3000 rcf for 5 minutes.

i. Aspirate hexane to waste using a Pasteur pipette on a hose connected to a side-arm Erlenmeyer flask.

j. Pipet a 5 mL aliquot of the extract into a pre-labeled 15 mL polypropylene centrifuge tube.

k. Evaporate extract to ≤ 0.5 mL in TurboVap at 45 ± 2°C at 15 psi setting, changing to 20 psi after 10 minutes. Total time to evaporate to ≤ 0.5 mL is 45 to 60 minutes.

l. Per the table below, add the volumes of the standard mixes and solutions to the matrix matched standards, add 140 µL acetonitrile to all other tubes, and dilute to 1.0 mL with 0.1% formic acid in water. Vortex all tubes for 5 seconds.

Note: After dilution to 1.0 mL with 0.1% formic acid in water, the extracts will contain ≈14% acetonitrile by volume.

Table 15 – Preparation of matrix matched standards

Sample Type	Acetonitrile Standard Mix (µL)	Beta Lactam Standard Mix (µL)	Internal Standard Mix (µL)	Acetonitrile (µL)
Samples, Recoveries, and Negative Controls				140
1/2X Matrix Matched Standard	20	20	20	100
1X Matrix Matched Standard	40	40	20	80
2X Matrix Matched Standard	80	80	20	40
3X Matrix Matched Standard	120	120	20	0

Note: If no internal standard mix is used, the volume of acetonitrile added to each tube must be increased by 20 µL.

m. Pipet 500 µL of all final extracts and calibration standards into bottom portions of pre-labeled Whatman Mini UniPrep Syringless Filter Vials, PVDF, 0.2 micron.

n. Press filter/caps onto the vials and place in autosampler tray for UHPLC-MS/MS analysis.

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

a. Instrument Operating Parameters - UHPLC system

i. Mobile phase for Residue analysis:

Mobile Phase A - 95% water / 5% ACN / 0.1% Formic Acid
Mobile Phase B - 100% ACN / 0.1% Formic Acid

Flush column with 1:1 A/B at a flow rate of 0.5 mL/min for three minutes. Change the mobile phase initial conditions to 100% A. Allow column to equilibrate until the "delta" value on the pressure reading is < 20.

ii. UHPLC gradient program:

Flow rate: 0.5 mL/min
Pressure Limits: 200 psi minimum; 15,000 psi maximum
Run time: 12.9 minutes

Table 16 – UHPLC gradient program

Time (min)	*% Mobile Phase A*	*% Mobile Phase B*	*Gradient*
0.00	100	0	none
0.10	100	0	linear
8.00	0	100	linear
9.50	0	100	linear
9.60	100	0	linear
12.90	100	0	linear

b. Autosampler program:

i. Run time: 12.90 minutes

ii. Injection loop: 20 µL

iii. Loop option: Full Loop

iv. Injection volume: 20 µL

v. Weak wash solvent: 10/90 Acetonitrile/Water

vi. Weak wash volume: 1200 µL

vii. Strong wash solvent: 50/50 Acetonitrile/Water

viii. Strong wash volume: 400 µL

ix. Sample temperature: 10 °C

c. Column manager

i. Column valve position: To match column location.

ii. Column manager temperature: 40 °C

iii. Use divert valve to divert eluant to waste 0.25 minutes prior to first peak and 0.25 minutes after last analyte peak.

d. Instrument Operating Parameters - Mass Spectrometer

Mass Spectrometer calibration and resolution are to be done according to the manufacturer's specification using the manufacturer's supplied calibration solution.

i. Type: MS/MS

ii. Electrospray Source Parameters:

(a) Capillary (kV): 3.0

(b) Cone (V): Variable - analyte dependent

(c) Extractor (V): 3.0

(d) RF (V): 0.10

(e) Source Temperature (°C): 150

(f) Desolvation Temperature (°C): 450

(g) Cone Gas Flow (L/hr): 20

(h) Desolvation Gas Flow (L/hr): 900

(i) Collision Gas Flow (mL/min): 0.10

iii. Analyzer Parameters:

(a) LM 1 Resolution: 10.6

(b) HM 1 Resolution: 14.8

(c) MSMS Mode Entrance: 1

(d) MSMS Mode Collision Energy: Variable – analyte dependent

(e) MSMS Mode Exit: 0.5

(f) LM 2 Resolution: 9.5

(g) HM 2 Resolution: 15.8

iv. MS Method Parameters:

(a) Type: MRM

(b) Ion Mode: ES+

(c) Dwell (s): 0.005

(d) Start time (min): 0.4

(e) End time (min): 6.1

v. Instrumental Settings - Scan Parameters

Table 17 – Instrument settings

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
2-Quinoxaline Carboxylic Acid	2.35	2.22-2.60	5	174.95	**129.00** 131.00 102.00	16 16 30	22
Amoxicillin	1.45	1.25 – 1.90	5	366.14	114.00 **349.34** 208.09	22 10 14	20
Salbutamol	1.45	1.27 - 1.67	5	240.23	**148.18** 222.30 166.15	20 10 15	20
Cimaterol	1.50	1.32 - 1.72	5	220.04	**142.97** 115.88 88.91	24 16 10	20
DCCD	1.65	1.55 - 2.10	5	549.10	**182.96** 241.12 125.91	30 20 76	40
Lincomycin	1.84	1.69 - 2.09	5	407.31	**126.10** 359.22 389.21	35 20 15	40
Sulfadiazine	1.91	1.79 - 2.19	5	251.10	**156.08** 108.02 158.08	15 20 15	30
Ampicillin	1.98	1.82 - 2.35	5	350.14	**106.07** 114.00 160.07	24 30 24	26
Desethylene Ciprofloxacin	2.03	1.88 - 2.30	5	306.20	**288.19** 245.18 289.28	20 20 10	35
Sulfathiazole	2.01	1.90 - 2.30	5	256.07	**156.08** 108.04 101.03	15 25 25	25
Sulfapyridine	2.07	1.92 - 2.37	5	250.09	**156.08** 92.00 108.11	18 26 28	32

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Norfloxacin	2.12	1.95 - 2.43	5	320.17	**276.19**	20	35
					233.10	30	
					219.03	20	
Tulathromycin A	2.16	1.93 - 2.38	5	806.75	**72.01**	35	40
					577.47	25	
					116.04	30	
Oxytetracycline	2.17	1.94 - 2.40	5	461.23	**426.35**	20	25
					443.39	15	
					201.24	25	
Ciprofloxacin	2.18	2.00 - 2.45	5	332.23	**245.18**	25	35
					288.39	20	
					203.21	40	
Ractopamine	2.25	2.08 - 2.48	5	302.20	**164.03**	15	20
					106.98	30	
					120.98	30	
Sulfamerazine	2.22	2.10 - 2.49	5	265.04	**91.88**	30	35
					155.92	15	
					107.91	15	
Danofloxacin	2.27	2.12 - 2.52	5	358.12	**96.03**	30	30
					314.18	15	
					283.14	25	
Tetracycline	2.32	2.16 - 2.56	5	445.23	**154.13**	30	30
					410.22	20	
					427.32	15	
Enrofloxacin	2.35	2.20 - 2.60	5	360.24	**316.40**	20	35
					245.25	25	
					203.16	40	
Sulfamethizole	2.44	2.30 - 2.70	5	271.06	**156.08**	16	28
					91.97	30	
					107.98	26	
Sulfamethazine	2.47	2.26 - 2.65	5	279.14	**186.14**	20	35
					156.08	20	
					108.12	25	
Sulfamethazine-6C13	2.47	2.24 - 2.73	5	285.17	**186.06**	18	32
					98.02	32	
					124.05	26	
Cefazolin	2.44	2.32 - 2.80	5	455.13	**156.03**	16	20
					323.15	12	
					112.08	35	
Sulfamethoxypyridazine	2.49	2.34 - 2.72	5	281.12	**156.09**	20	30
					126.17	20	
					108.06	25	

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Difloxacin	2.59	2.27 - 2.75	5	400.25	**356.43** 299.23 285.28	20 30 40	35
Sarafloxacin	2.55	2.35 - 2.75	5	386.09	**342.18** 299.17 270.20	20 25 45	40
Pirlimycin	2.74	2.44 - 2.84	5	411.29	**112.20** 363.33 110.34	40 20 45	30
Chlortetracycline	2.81	2.63 - 3.03	5	479.19	**154.08** 444.26 462.20	30 20 20	30
Clindamycin	2.87	2.68 - 3.08	5	425.30	**126.22** 377.40 124.32	40 20 45	45
Gamithromycin	2.94	2.70 - 3.10	5	777.79	**83.02** 115.97 158.10	48 36 46	66
Sulfachloropyridazine	2.85	2.29 - 3.00	5	285.00	**156.08** 107.98 91.97	16 26 30	28
Tilmicosin	3.10	2.83 - 3.25	5	869.79	**174.16** 132.23 696.63	35 35 35	45
Sulfadoxine	3.00	2.85 - 3.25	5	311.15	**156.08** 108.14 140.09	20 30 30	35
Sulfamethoxazole	3.00	2.89 - 3.29	5	254.02	**92.06** 155.97 107.89	25 20 20	30
Sulfaethoxypyridazine	3.04	2.91 - 3.31	5	295.13	**156.09** 140.18 108.09	20 20 25	30
Florfenicol	3.06	2.93 - 3.33	5	358.10	**241.01** 206.00 130.36	18 22 60	20
Chloramphenicol	3.25	3.00 - 3.60	5	321.12	**152.13** 194.27 257.13	22 10 10	34
Erythromycin A	3.53	3.28 - 3.68	5	734.75	**158.15** 115.89 576.48	30 40 20	30

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Sulfadimethoxine	3.46	3.33 - 3.75	5	311.14	**156.10** 108.04 245.22	20 30 20	35
Sulfaquinoxaline	3.47	3.35 - 3.75	5	301.10	**156.13** 107.98 91.97	18 28 36	34
Prednisone	3.56	3.37 - 3.85	5	359.15	**341.11** 146.94 267.28	10 26 15	22
Tylosin	3.70	3.46 - 3.86	5	916.76	**174.20** 101.08 145.15	35 35 35	45
Pen G-d7	3.74	3.63 - 4.20	5	342.13	**183.10** 160.10 98.10	26 24 54	46
Penicillin G	3.76	3.61 - 4.03	5	335.13	**176.02** 160.10 114.01	16 18 30	18
Sulfanitran	4.05	3.93 - 4.33	5	336.15	**156.08** 134.13 92.71	14 28 38	30
Taleranol (β-Zearalanol)	4.26	4.13 - 4.53	5	323.21	**305.2** 189.09 149.02	10 24 30	16
Oxacillin	4.29	4.17 - 4.70	5	402.14	**160.00** 243.08 144.06	20 18 34	22
Cloxacillin	4.55	4.43 - 5.05	5	436.16	**160.06** 277.14 114.07	12 16 44	22
Nafcillin	4.68	4.55 - 5.10	5	415.22	**199.17** 171.07 115.11	16 42 70	20
Oxyphenylbutazone	4.74	4.59 - 4.99	5	325.18	**120.06** 148.16 204.12	24 30 16	26
Flunixin	4.82	4.61 - 5.03	5	297.05	**279.05** 109.00 264.04	22 50 32	42
Flunixin-d3	4.81	4.63 - 5.03	5	300.05	**282.12** 112.03 264.04	24 54 36	40

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Dicloxacillin	4.93	4.80 - 5.35	5	470.19	**160.06** 311.11 114.07	14 16 48	22
Phenylbutazone	5.85	5.69 - 6.09	5	309.12	**119.95** 76.90 91.80	22 24 18	32
Melengestrol acetate	6.18	6.04 - 6.44	5	397.35	**279.33** 337.46 221.28	20 15 40	30
2-Aminosulfone Albendazole	1.90	1.35 - 2.05	5	240.04	105.55 **132.96** 197.96	46 28 20	36
2-amino-Flubendazole	2.81	2.49 - 3.19	5	256.05	94.86 **122.87** 132.90	38 28 36	50
Abamectin	7.93	7.51 - 8.21	5	890.84	112.96 144.99 **305.26**	46 42 28	16
Acepromazine	3.63	3.29 - 3.99	5	327.24	**85.97** 222.07 254.03	20 42 22	32
Albendazole	3.67	3.32 - 4.02	5	266.12	158.90 191.12 **234.04**	38 32 20	34
Azaperone	2.41	2.06 - 2.76	5	328.27	94.86 122.93 **165.00**	62 36 20	34
Butorphanol	2.97	2.62 - 3.32	5	328.19	124.13 157.14 **310.24**	28 46 22	10
Carazolol	2.96	2.61 - 3.31	5	299.26	97.92 **115.96** 222.05	22 20 22	34
Diclofenac	5.53	5.14 - 5.84	5	296.05	**214.93** 250.02 278.01	20 12 10	20

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Dimetridazole	1.88	1.52 - 2.22	5	141.96	80.88 **95.87** 111.90	24 16 12	32
Dimetridazole - OH	1.65	1.30 - 2.00	5	158.02	93.90 **111.88** 139.97	22 20 12	22
Dipyrone	1.61	1.28 - 1.98	5	218.09	**96.94** 124.96 187.04	12 12 10	24
Doramectin	8.28	7.87 - 8.57	5	916.85	112.95 145.00 **331.27**	56 48 26	22
Doxycycline	2.93	2.58 - 3.28	5	445.31	97.90 153.90 **428.20**	46 32 20	28
Emamectin Benzoate	5.69	5.36 - 6.06	5	886.81	125.98 **158.02** 302.20	46 40 28	52
Eprinomectin	7.41	7.05 - 7.75	5	914.80	112.05 154.05 **186.08**	74 40 20	18
Fenbendazole	4.32	3.96 - 4.66	5	300.11	130.94 158.94 **268.01**	46 36 20	38
Fenbendazole sulphone	3.62	3.30 - 4.00	5	332.10	130.94 158.94 **300.04**	50 38 22	40
Flubendazole	3.88	3.51 - 4.21	5	314.12	94.85 122.93 **282.05**	50 28 22	38
Haloperidol	3.62	3.28 - 3.98	5	376.24	94.92 122.93 **165.00**	68 42 24	40
Ipronidazole	3.21	2.83 - 3.53	5	170.05	95.84 109.00 **123.99**	22 24 18	34

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Ipronidazole - OH	2.53	2.17 - 2.87	5	186.05	121.84 127.90 **167.98**	20 16 14	22
Ketamine	2.29	1.97 - 2.67	5	239.18	126.03 180.10 **221.14**	22 16 16	34
Ketoprofen	4.68	4.30 - 5.00	5	255.09	76.84 104.85 **209.00**	40 24 14	28
Levamisole	1.91	1.56 - 2.26	5	205.04	90.88 122.93 **177.96**	34 28 22	40
Meloxicam	4.86	4.48 - 5.18	5	352.00	**114.91** 140.94 184.02	20 24 16	30
Metronidazole	1.63	1.29 - 1.99	5	171.96	81.88 110.89 **127.89**	24 24 14	26
Metronidazole - OH	1.44	1.09 - 1.79	5	188.03	122.96 **125.95** 143.92	14 18 14	22
Morantel tartrate	2.66	2.31 - 3.01	5	221.07	110.91 **122.89** 150.04	24 36 28	50
Moxidectin	8.29	7.86 - 8.56	5	640.53	199.03 498.22 **528.34**	22 10 8	16
Orbifloxacin	2.41	2.07 - 2.77	5	396.24	295.07 **352.15** 378.18	24 18 20	36
Promethazine	3.66	3.31 - 4.01	5	285.16	**85.94** 197.97 240.09	16 20 16	24
Propionylpromazine	4.00	3.67 - 4.37	5	341.19	**85.93** 236.04 268.07	22 40 24	32

Analyte	RT (min)	window (min)	Dwell Times (ms)	Precursor Ion(m/z)	Product Ions (m/z)	Collision Energy (V)	Cone (V)
Ronidazole	1.85	1.50 - 2.20	5	201.02	54.84 66.89 **139.93**	20 32 10	18
Selamectin	8.57	8.14 - 8.84	5	770.66	94.92 112.96 **144.99**	52 40 30	36
Thiabendazole	1.95	1.60 - 2.30	5	202.00	64.88 130.94 **174.91**	40 32 26	44
Tildipirosin	1.74	1.37 - 2.07	5	734.62	115.70 132.02 **174.06**	56 46 38	58
Tolfenamic Acid	6.18	5.77 - 6.47	5	262.09	180.00 228.97 **244.01**	40 32 14	22
Tyvalosin	4.76	4.44 - 5.14	5	1043.76	83.16 **109.28** 174.13	60 48 36	62
Virginiamycin	4.45	4.09 - 4.79	5	526.40	108.90 355.10 **508.30**	44 18 12	26
Xylazine	2.66	2.18 - 2.88	5	221.07	90.01 105.02 **147.04**	22 36 24	42
Chlorpromazine	4.12	3.8 - 4.5	5	319.19	**85.97** 213.94 245.97	20 42 22	32
Triflupromazine	4.35	4.04 - 4.74	5	353.21	**85.94** 248.02 280.05	22 44 28	34

Note: Product ions are listed with the expected screening ion in **bold** along with diagnostic ions 1 and 2.

Note: The following parameters will be used if screening for Chloramphenicol in negative electrospray ionization mode:

Analyte	RT	Window	Dwell Time	Precursor	Product	Collision	Cone
ES (-)	(min)	(min)	(ms)	Ion (m/z)	Ions (m/z)	Energy (V)	(V)
Chloramphenicol	3.25	3.00-3.60	0.008	321.09	152.03	15	35

4. Sample Set
    a. Screening Set
        i. External Standard(s) (optional)
        ii. Matrix matched standard
        iii. Recovery(ies) (positive controls)
        iv. Check sample (if necessary)
        v. Blank (negative control)
        vi. Up to 27 Samples
        vii. External standard, matrix matched standard, or recovery
    b. Confirmation Set
        i. External Standard(s) (optional)
        ii. Matrix matched standards
        iii. Recovery(ies) (positive control)
        iv. Check sample (if necessary)
        v. Matrix Matched Blank (negative control)
        vi. Up to 24 Samples
        vii. External standard, matrix matched standard, or recovery

Note: Placing solvent blanks in the sample injection sequence is prudent in case a high finding leads to carry-over. Additionally, one may want to include an additional external standard, matrix matched standard, or recovery within the sample injection sequence to verify retention time and instrument response stability.

G. CALCULATIONS / IDENTIFICATION

1. Screening
    a. The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 17.
    b. The retention times for the screening ion in the fortified recoveries must match the retention time of the screening ion in the matrix-matched standard within 5%. Retention time for the screening ions in the samples must match the retention time of the screening ions in a fortified recovery or the matrix matched standard within 5%.

c. The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored daughter ions.

d. A sample is screened positive for an analyte if the following criteria are met:

i. The fortified recovery of the analyte must exceed 10% of the 1/2 X matrix matched standard level.

ii. The sample response equals or exceeds the 1/2 X (or level of interest) fortified recovery level.

e. The level of the screening ion in the blank (negative control) must be less than 10% of the 1/2 X level for the matrix-matched standard.

Note: If a sample shows a positive response for a compound which did not meet screening criteria in the associated QC samples, then further testing of that sample is warranted.

2. Confirmation

a. Monitored ions for each analyte will be assessed as follows:

i. Recovery retention times must match the retention time of the matrix matched standard within 5%. Retention time for the samples must match the retention time of the positive control or the matrix matched standard within 5%.

ii. All product ions specified for ratio matching are present with a signal-to-noise ratio ≥ 3. This may be verified by visual inspection. Visual inspection for detection may also include assessment of peak shape or drift in relation to standard peaks or evaluating the presence or absence of monitored secondary or tertiary ions.

iii. One of the following ion ratio matching conditions is met:

Note: Ratios are calculated by dividing the area count of each diagnostic ion by the area count of the base ion. Ion ratios should be less than 1. If the ratio is not less than 1 for a sample set, the inverse of this ratio may be used.

(a) If two product ions are assessed, one sample ion ratio should match the calculated average ratio of the matrix-matched standards within a ± 10% absolute difference.

(b) If three product ions are monitored, the presence of two sample ion ratios should match the calculated average ratio of the matrix-matched standards within a ± 20% absolute difference.

b. A sample is confirmed positive for an analyte if the following criteria are met:

i. The fortified recovery of the analyte must exceed 10% of the 1/2 X matrix matched standard level.

ii. The sample response equals or exceeds the 1/2 X (or level of interest) fortified recovery level.

c. The blank (negative control) must be less than 10% of the 1/2 X level for the matrix matched standard.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Required Protective Equipment -Safety eyewear, protective gloves, and lab coat.

2. Hazards

Procedure Step	*Hazard*	*Recommended Safe Procedures*
Antibiotic standards	Some individuals may have allergic reactions to certain β-lactams, sulfa, or other drugs.	Wear appropriate personal protective equipment to avoid dermal contact.
Acetonitrile, Methanol	Flammable	Keep in well-closed containers away from ignition sources. Avoid contact or prolonged exposure to vapors. Work in fume hood. Keep away from flame or heat.
Formic acid	Corrosive, Caustic	Wear personal protective equipment, avoid skin contact.

3. Disposal Procedures

Follow local, state and federal guidelines for disposal.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

a. Screening Criteria

i. For set acceptance, 90% of the monitored analytes in the fortified recovery (positive control) must meet screening criteria. For sample

reporting purposes, the analytes of interest in the fortified recovery (positive control) must meet screening criteria.

ii. For set acceptance, 90% of the monitored analytes in the blank (negative control) must not meet screening criteria. The blank (negative control) must be negative using the criteria in Section G for samples containing corresponding presumptive positive analytes.

b. Confirmation Criteria

i. For set acceptance, nine of the following ten analytes must meet confirmation criteria (Dicloxacillin, Enrofloxacin, Erythromycin A, Florfenicol, Flunixin, Oxytetracycline, Penicillin G, Meloxicam, Sulfadimethoxine, and Fenbendazole Sulphone). For sample reporting purposes, the analytes of interest in the fortified recovery (positive control) must meet confirmation criteria.

ii. The blank (negative control) must be negative using the criteria in Section G for the analytes of interest.

2. Critical Control Points and Specifications

Record	*Acceptable Control*
none known at this time	

3. Intralaboratory Check Samples

a. System, minimum contents.

i. Frequency: One per week per analyst when samples analyzed.

ii. Records are to be maintained.

b. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Investigate following established procedures.

ii. Take corrective action as warranted.

4. Sample Condition upon Receipt

Cool or frozen

J. APPENDIX

1. References

   [Reserved]

2. Chromatograms/spectra

   [Reserved]

3. Minimum Level of Applicability

Table 18A - Minimum Level of Applicability for kidney screening

Standard Analyte	Minimum Level of Applicability for kidney screening Kidney Screening Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	25	25	25	25	50
2-amino-Flubendazole	10	40	20	20	10
2-Quinoxaline Carboxylic Acid (QCA)			30		60
Abamectin	25	50	25	25	25
Acepromazine	4	4	4	1	1
Albendazole	25	25	25	25	25
Ampicillin	10	10		5	20
Azaperone	4	1			
Butorphanol	4	1		2	4
Carazolol	1	1	4	2	1
Cefazolin	50	50	50	50	50
Chloramphenicol	6	6	6	6	6
Chlorpromazine					2
Chlortetracycline	1000	1000	1000	1000	1000
Ciprofloxacin	25	25		25	100
Clindamycin	50	50	50	50	50
Cloxacillin	10	5	5	5	5
Danofloxacin		25	50	25	25
DCCD	200	100	200	50	200
Desethylene Ciprofloxacin	25	25	100	25	50
Diclofenac	5	5	5	10	5
Dicloxacillin	50	50	50	50	50
Difloxacin	25	25	25	25	25
Dimetridazole - OH	200				
Doramectin	30		30		
Doxycycline	25	25	25	25	
Emamectin Benzoate	30	30	7.5	7.5	7.5
Enrofloxacin	25	25	25	25	25
Eprinomectin	15	30	30	30	
Erythromycin A	50	50	50	50	50
Fenbendazole	200	200	200	200	200

Standard Analyte	Minimum Level of Applicability for kidney screening Kidney Screening Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Fenbendazole sulphone	200	200	200	200	200
Florfenicol	100	100	100	100	100
Flubendazole	10	10	10	10	10
Flunixin	12.5	12.5	12.5	12.5	12.5
Gamithromycin	50	50	50	50	
Haloperidol	1	1	2	1	1
Ipronidazole	2	4		2	4
Ipronidazole - OH		4			
Ketamine		40			
Ketoprofen	5	5	20	20	20
Levamisole	50	50	50	50	50
Lincomycin	50	50	50	50	50
Melengestrol Acetate	20	20	20	40	40
Meloxicam	10	10	10	10	10
Metronidazole	2	4	4	4	
Metronidazole - OH	4	4	4	8	16
Morantel tartrate	350	350	350	350	350
Moxidectin	30		7.5	30	
Nafcillin	50	50	50	50	50
Norfloxacin	25	25	25	100	25
Orbifloxacin	25	25	25	25	25
Oxacillin	50	50	50	50	50
Oxyphenylbutazone	100	50			
Oxytetracycline	1000	1000	500	500	500
Penicillin G	25	25	25	25	25
Phenylbutazone	50	50			
Pirlimycin	250	250	250	250	250
Prednisone	200	50		100	
Promethazine	4	4		4	4
Propionylpromazine	4	4		1	1
Ractopamine	15	15	15	15	15
Salbutamol	3	3	3	3	3
Sarafloxacin	25	25	25	25	25
Sulfachloropyridazine	50	50	50	50	50
Sulfadiazine	50	50	50	50	50
Sulfadimethoxine	50	50	50	50	50
Sulfadoxine	50	50	50	50	50
Sulfaethoxypyridazine	50	50	50	50	50
Sulfamerazine	50	50	50	50	50
Sulfamethazine	50	50	50	50	50
Sulfamethizole	50	50	50	50	50
Sulfamethoxazole	50	50	50	50	50
Sulfamethoxypyridazine	50	50	50	50	50

Standard Analyte	Minimum Level of Applicability for kidney screening Kidney Screening Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Sulfanitran	50	200	200	200	50
Sulfapyridine	50	50	50	50	50
Sulfaquinoxaline	50	50	50	50	50
Sulfathiazole	50	50	50	50	50
Tetracycline	500	500	500	500	500
Thiabendazole	50	50	50	50	50
Tildipirosin	2000	2000	500	500	
Tilmicosin	60	240	60	60	
Tolfenamic Acid	25	50	100	50	25
Triflupromazine				4	
Tulathromycin A	1000	4000	1000		
Tylosin	100	100	100	100	100
Tyvalosin	25	25	25	25	25
Virginiamycin	50	50	50	50	50
Xylazine		1	4		

Table 18B - Minimum Level of Applicability for kidney confirmation

Standard Analyte	Minimum Level of Applicability for kidney confirmation Kidney Confirmation Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
2-Aminosulfone Albendazole	25	50	25		100
2-amino-Flubendazole	10	40	40	20	20
2-Quinoxaline Carboxylic Acid (QCA)			30		60
Abamectin	25	50	25	50	100
Acepromazine					4
Albendazole	25	25	25	25	25
Ampicillin	10	10		20	20
Butorphanol		2		4	4
Carazolol	1	1	4	2	2
Cefazolin	100	100	100	100	50
Chlortetracycline	1000	1000	1000	1000	1000
Ciprofloxacin				50	
Clindamycin	50	50	50	50	50
Cloxacillin	10	20		10	20
Danofloxacin			50	25	100
DCCD	200	200	200	100	
Dicloxacillin	50	50	50	50	50
Difloxacin	25	25	25	25	25
Doxycycline	25				
Emamectin Benzoate	30	30	7.5	7.5	15
Enrofloxacin	25	25	25	50	25
Eprinomectin	30				

Standard Analyte	Minimum Level of Applicability for kidney confirmation Kidney Confirmation Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Erythromycin A	50	50	50	50	50
Fenbendazole	200	200	200	200	200
Fenbendazole sulphone	200	200	200	200	200
Florfenicol	100	100	100	100	100
Flubendazole	10	10	20	10	10
Flunixin	12.5	12.5	12.5	12.5	12.5
Gamithromycin	50		50	50	
Haloperidol	2	2		1	4
Ipronidazole		4			
Ketoprofen	5	5	20	20	20
Levamisole	50	200	50	200	200
Lincomycin	50	50	50	50	50
Melengestrol Acetate	20	20	20	40	40
Meloxicam	10	10	10	10	10
Morantel tartrate	350	350	350	350	1400
Moxidectin	30			30	
Nafcillin	50	50	50	50	50
Norfloxacin	100	25	100	100	25
Orbifloxacin	25	100			
Oxacillin	50	50	50	50	50
Oxyphenylbutazone	200				
Oxytetracycline	1000	1000	500	500	500
Penicillin G	25	25	25	50	50
Phenylbutazone	50	50			
Pirlimycin	250	250	250	250	250
Prednisone	200	200			
Promethazine		4			4
Ractopamine	15				
Salbutamol	3	3	6	6	3
Sarafloxacin	25	50	50	100	25
Sulfachloropyridazine	50	50	50		50
Sulfadiazine	50	50	50	50	50
Sulfadimethoxine	50	50	50	50	50
Sulfadoxine	50	50	50	50	50
Sulfaethoxypyridazine	50	50	50	50	50
Sulfamerazine	50	50	50	50	50
Sulfamethazine	50	100	50	200	200
Sulfamethizole	50	200	100	50	50
Sulfamethoxazole	50	50	200	50	200
Sulfamethoxypyridazine	50	200	50	50	50
Sulfanitran	50	200	200	200	50
Sulfapyridine		50	200	100	
Sulfaquinoxaline	50	50	50	50	50

Standard Analyte	Minimum Level of Applicability for kidney confirmation Kidney Confirmation Level (ng/g)				
	Bovine	Porcine	Poultry	Caprine	Ovine
Sulfathiazole	50	50	50	50	50
Tetracycline	2000	500	500	500	500
Thiabendazole	50	50	100	50	50
Tildipirosin	2000	2000	500	500	
Tilmicosin	60	240	60	60	
Tolfenamic Acid	50	100	100	50	25
Tulathromycin A	1000	4000			
Tylosin	100	100	100	100	100
Tyvalosin	25	25	25	25	25
Virginiamycin	50	50	50	50	50

Table 19A - Minimum Level of Applicability for muscle screening and confirmation

Standard Analyte	Minimum Level of Applicability (MLA), Muscle Screening All Species (ng/g)	Minimum Level of Applicability (MLA) Muscle Confirmation (ng/g)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
2-Aminosulfone Albendazole	25	50	25	25	100	100	25	50
2-amino-Flubendazole	10	20	10	10	20	20	40	40
2-Quinoxaline Carboxylic Acid (QCA)	15 [A]			30	30	30	60	30
Abamectin	25 [J]		100			100		
Acepromazine	2 [B]							
Albendazole	25 [I]	25	50		50	25	25	25
Amoxicillin	80 [K]							
Ampicillin	5 [E]	10		5		10		10
Azaperone	1		4					
Butorphanol	1	1	2	2		4		
Carazolol	1	1	1	1	1	2	2	4
Cefazolin	50	100	100	100	100	200	50	200
Chloramphenicol	3 [D]							
Chlortetracycline	1000	4000	1000	1000		1000	2000	4000
Cimaterol	3							
Ciprofloxacin	25	100	100	50			100	100
Clindamycin	50	50	50	50	50	50	50	50
Cloxacillin	5	10	10	10	5	5		10
Danofloxacin	25		50	25	25	25		25
DCCD	50 [H]	100	200	200	200		200	200
Desethylene Ciprofloxacin	25							
Diclofenac	5							20
Dicloxacillin	50	50	50	50	50	50	50	50
Difloxacin	25	25	25	25	25	25	25	100
Dimetridazole	2 [O]							
Dimetridazole - OH	50	50	50	50	50	100	200	200

Standard Analyte	Minimum Level of Applicability (MLA), Muscle Screening All Species (ng/g)	Minimum Level of Applicability (MLA) Muscle Confirmation (ng/g)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Dipyrone	25 [C]		50					25
Doramectin	30 [M]							
Doxycycline	25 [H]		100					
Emamectin Benzoate	7.5 [P]		30		15			15
Enrofloxacin	25	25	25	25	50	25	25	25
Eprinomectin	30 [Q]							
Erythromycin A	50	50	50	50	50	100	50	50
Fenbendazole	200	200	400	200	200	200	200	200
Fenbendazole sulphone	200	200	200	200	200	200	200	200
Florfenicol	100	100	100	100	100	100	100	100
Flubendazole	10	10	10	10	10	10	10	10
Flunixin	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5
Gamithromycin	50	50	100	200	50	50		100
Haloperidol	1	1	2	1	2	1	2	1
Ipronidazole	1 [G]		4					
Ipronidazole - OH	1 [L]							
Ketamine	20 [R]							
Ketoprofen	5 [E]	5	5	5	10	5		10
Levamisole	50	50	50	50	50	50	200	
Lincomycin	50	50	50	50	50	50	50	50
Melengestrol Acetate	20 [H]	20	40	20	80		20	20
Meloxicam	10	10	10	10	10	10	20	10
Metronidazole	1							
Metronidazole - OH	4							
Morantel tartrate	350	350	350	350	350	350		
Moxidectin	30 [S]		30					
Nafcillin	50	50	50	50	50	50	50	50
Norfloxacin	25	25	25	25	25	50	100	100
Orbifloxacin	25		25	100	50	50	100	
Oxacillin	50	50	50	50	50	50	50	50
Oxyphenylbutazone	50 [C]							50
Oxytetracycline	500	500	500	500	500	500	500	500
Penicillin G	25	25	25	25	25	25	50	100
Phenylbutazone	50 [C]		100					50
Pirlimycin	250	250	250	250	250	250	250	250
Prednisone	50	50	100	50	100	100	200	100
Ractopamine	15	15	15	15	15			
Ronidazole	1 [O]							
Salbutamol	3	3	3	3	3	6	3	3
Sarafloxacin	25	25	25	100	50	25	100	
Selamectin	30 [M]							
Sulfachloropyridazine	50	50	50	200	50	50	100	

Standard Analyte	Minimum Level of Applicability (MLA), Muscle Screening All Species (ng/g)	Minimum Level of Applicability (MLA) Muscle Confirmation (ng/g)						
		Bovine	Porcine	Poultry	Caprine	Ovine	Equine	Siluriformes
Sulfadiazine	50	50	50	50	50	50	50	100
Sulfadimethoxine	50	50	50	50	50	50	50	50
Sulfadoxine	50	50	50	50	50	50	50	50
Sulfaethoxypyridazine	50	100	50	50	50	50	50	50
Sulfamerazine	50	50	50	50	50	50	100	100
Sulfamethazine	50		50		100	50	200	
Sulfamethizole	50	50	100	50	50	50	50	200
Sulfamethoxazole	50	50	50	50	100	50	50	50
Sulfamethoxypyridazine	50	50	50	50	50	200	100	
Sulfanitran	50	50	50	100	50	50	100	50
Sulfapyridine	50	50	50	50	50	50		
Sulfaquinoxaline	50	50	50	50	50	50	50	50
Sulfathiazole	50	50	50	50	50	50	50	50
Tetracycline	500	500	500		500	500	1000	2000
Thiabendazole	50	50	50	50	100	50		
Tildipirosin	500	500	500	500	500	500	500	500
Tilmicosin	60	60	60	60	60	60	60	60
Tolfenamic Acid	25	50	50	50	100	50	50	100
Tulathromycin A	1000 [F]	2000	1000	2000		1000	2000	4000
Tylosin	100	100	100	100	100	100	100	100
Tyvalosin	25	25	50	25	25	25	25	25
Virginiamycin	50	50	50	50	50	50	50	50
Xylazine	1 [N]							
Taleranol(β-Zearalanol)	12 [B]							24

A – Not applicable for bovine or porcine
B – Applicable for Siluriformes only
C – Not applicable for bovine, poultry, caprine, ovine or equine
D – Not applicable for Siluriformes
E – Not applicable for equine
F – Not applicable for caprine
G – Not applicable for equine or Siluriformes
H – Not applicable for ovine
I – Not applicable for poultry
J – Not applicable for bovine, poultry, caprine or Siluriformes
K – Not applicable for porcine, poultry, caprine, ovine or equine
L – Not applicable for ovine or equine
M – Applicable for porcine only
N – Not applicable for bovine or ovine
O – Not applicable for caprine, ovine or equine
P – Not applicable for bovine, poultry or equine
Q – Not applicable for bovine, caprine, equine or Siluriformes
R – Not applicable for poultry, caprine, ovine or equine

S – Not applicable for bovine, poultry, equine or Siluriformes

K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Staff.